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**United States Patent** [19]

Billiar et al.

[11] **Patent Number:** 5,658,565[45] **Date of Patent:** Aug. 19, 1997[54] **INDUCIBLE NITRIC OXIDE SYNTHASE GENE FOR TREATMENT OF DISEASE**

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[58] **Field of Search** ..... 435/189, 191, 435/320.1, 235.1; 514/44; 424/423, 93.1, 93.2; 536/23.1, 23.2, 23.5; 935/9, 10, 14, 22, 23, 32

[56] **References Cited****U.S. PATENT DOCUMENTS**

5,132,407	7/1992	Shuehr et al.	530/395
5,252,479	10/1993	Strivastava	435/320.1
5,328,470	7/1994	Nabel et al.	604/101
5,428,070	6/1995	Cooke et al.	514/557

**FOREIGN PATENT DOCUMENTS**

92/07943 5/1992 WIPO.

**OTHER PUBLICATIONS**

Geller, et al., 1993, Molecular Cloning and Expression of Inducible Nitric Oxide Synthase from Human Hepatocytes, PNAS 90: 3491-3495.

Nussler, et al., 1992, Stimulation of the Nitric Oxide Synthase Pathway in Human Hepatocytes by Cytokines and Endotoxins, J. Exp. Med. 176: 261-264.

Lowenstein, et al., 1992, Cloned and Expressed Macrophage Nitric Oxide Synthase Contrasts with the Brain Enzyme, PNAS 89: 6711-6715.

Xie, et al., 1992, Cloning and Characterization of Inducible Nitric Oxide Synthase from Mouse Macrophages, Science 256: 225-228.

Lyons, et al., 1992, Molecular Cloning and Functional Expression of an Inducible Nitric Oxide Synthase from a Murine Macrophage Cell Line, J. Biol. Chem. 267: 6370-6374.

von der Leven, et al., 1994, *In Vivo* Gene Transfer to Prevent Neointima Hyperplasia after Vascular Injury: Effect of Overexpression of Constitutive Nitric Oxide Synthase, Faseb J. 8:A802 (#4651).

Bucala, et al., 1991, Advanced Glycosylation Products Quench Nitric Oxide and Mediate Defective Endothelium-Dependent Vasodilatation in Experimental Diabetes, J. Clin. Invest. 87: 432-438.

Chin, et al., 1992, Inactivation of Endothelial Derived Relaxing Factor by Oxidized Lipoproteins, J. Clin. Invest. 89:10-18.

Chester, et al., 1990, Low Basal and Stimulated Release of Nitric Oxide in Atherosclerotic Epicardial Coronary Arteries, Lancet 336: 897-900.

Wilson, et al., 1989, Implantation of Vascular Grafts Lines with Genetically Modified Endothelial Cells, Science 244: 1344-1346.

Ignarro, et al., 1987, Endothelium-Derived Relaxing Factor Produced and Released from Artery and Vein is Nitric Oxide, PNAS 84: 9265-9269.

Radomski et al., 1987, The Anti-Aggregating Properties of Vascular Endothelium: Interactions Between Prostacyclin and Nitric Oxide, Br. J. Pharmac. 92: 639-646.

Nunokawa and Tanaka, 1992, Interferon-Gamma Inhibits Proliferation of Rat Vascular Smooth Muscle Cells by Nitric Oxide Generation, Biochem. Biophys. Res. Comm. 188: 409-415.

Werner-Felmayer, et al., 1990, Tetrahydrobiopterin-Dependent Formation of Nitrite and Nitrate in Murine Fibroblasts, J. Exp. Med. 172: 1599-1607.

Moncada, et al., 1991, Nitric Oxide: Physiology, Pathophysiology and, Pharmacology, Pharmacological Reviews 43: 109-142.

Nabel, et al., 1989, Recombinant Gene Expression *In Vivo* Within Endothelial Cells of the Arterial Wall, Science 244: 1342-1344.

Zwiebel, et al., 1989, High-Level Recombinant Gene Expression in Rabbit Endothelial Cells Transduced by Retroviral Vectors, Science 243: 220-222.

Nabel, et al., 1990, Site-Specific Gene Expression *In Vivo* by Direct Gene Transfer into the Arterial Wall, Science 249:1285-1288.

Nussler, et al., 1992, Stimulation of Nitric Oxide in Human Hepatocytes by Cytokines, Faseb J., 6 (5): A1834 (#5200).

Draiper, et al., 1991, L-arginine-derived Nitric Oxide and the Cell-Mediated Immune Response, Res. Immunol. 142: 553-602.

Janssens, et al., 1992, Cloning and Expression of a cDNA Encoding Human Endothelial-Derived Relaxing Factor/Nitric Oxide Synthase, J. Biol. Chem. 267: 14519-14522.

(List continued on next page.)

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[57] **ABSTRACT**

The present invention discloses a full-length human hepatocyte iNOS cDNA clone and various gene therapy applications utilizing an iNOS DNA sequence. In preferred embodiments of the disclosed invention, iNOS-directed gene therapy involves specific targeting of a DNA sequence encoding a protein or protein fragment with iNOS biological activity for treating vascular diseases and disorders, antitumor applications and in response to certain microbial infections.

**40 Claims, 5 Drawing Sheets**